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**(54) Title: FORMULATIONS OF ANTHRAQUINONE DERIVATIVES**

$$\begin{array}{c}
 \text{OH} \quad \text{O} \quad \text{HN}-\overset{\text{O}^-}{\underset{\text{R}''}{\text{A}}} \text{N}^+ \text{R}' \\
 | \quad \quad | \quad \quad | \\
 \text{C}_6\text{H}_3\text{OH} \quad \text{C}_6\text{H}_3\text{O} \quad \text{C}_6\text{H}_3\text{NH}-\overset{\text{R}''}{\underset{\text{R}'}{\text{A}}} \text{N}^+ \text{O}^- \\
 | \quad \quad | \quad \quad | \\
 \text{OH} \quad \text{O} \quad \text{HN}-\overset{\text{R}''}{\underset{\text{R}'}{\text{A}}} \text{N}^+ \text{O}^- \\
 \end{array} \quad (1)$$

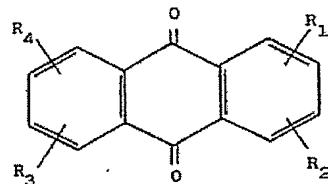
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**(57) Abstract:** A compound of formula (I): in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C<sub>1-4</sub> alkyl groups and C<sub>2-4</sub> hydroxyalkyl and C<sub>2-4</sub> dihydroxyalkyl groups, or R' and R" together are a C<sub>2-6</sub> alkylene group, is formulated so that upon dissolution in aqueous solution the pH of the solution is in the range of 5 to 9. The compound may be in the form of salt with a physiologically acceptable acid having a pK<sub>a</sub> in the range of -3.0 (minus 3.0) to 9.0.

## FORMULATIONS OF ANTHRAQUINONE DERIVATIVES

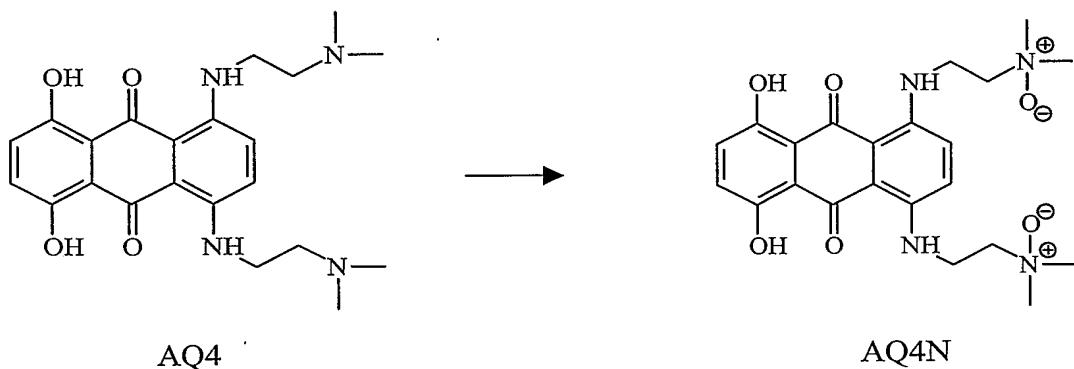
The invention relates to novel formulations of anthraquinone derivatives such as AQ4N, a bis-bioreductive agent with value in the treatment of cancer. It includes novel salt forms of the anthraquinone derivatives.

5 WO-A-91/05824 (National Research Development Corporation) discloses a compound of formula:



in which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are each separately selected from hydrogen, X, NH—A—NHR and NH—A—N(O)R'R" wherein X is hydroxy, halogeno, amino, C<sub>1-4</sub> alkoxy or C<sub>2-8</sub> alkanoyloxy, A is a C alkylene group with a chain length between NH and NHR or N(O)R'R" of at least 2 carbon atoms and R, R' and R" are each separately selected from C<sub>1-4</sub> alkyl groups and C<sub>2-4</sub> hydroxyalkyl and C<sub>2-4</sub> dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C<sub>2-6</sub> alkylene group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring, the compound optionally being in the form of a physiologically acceptable salt.

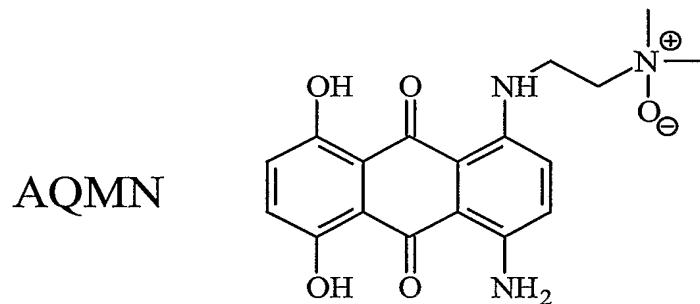
10 A preferred compound within this general formula is the *N*-oxide AQ4N, which would normally be synthesised by oxidation of AQ4:



20 AQ4N is in fact a prodrug and the reverse reaction occurs *in vivo*, reductive metabolism in hypoxic cancer cells giving the active agent, AQ4, in its protonated form. The prodrug is relatively non-toxic when compared with the active agent, AQ4,

making it particularly attractive for administration as a pharmaceutical. However, it does not readily give a crystalline form, and it is therefore desirable to prepare and formulate it for administration in the form of a salt.

5 AQ4N has up to now been reported in the form of a dihydrochloride salt AQ4N.2HCl. See for example *J. Chem. Soc., Perkin Trans. I*, 1999, 2755-2758 (Lee *et al.*) and WO-A-00/05194 (BTG International Limited). However, investigations of AQ4N.2HCl raw material have demonstrated significant quantities of an impurity 1-amino-4-{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthraquinone, denoted AQMN, which has been characterised by LCMS:

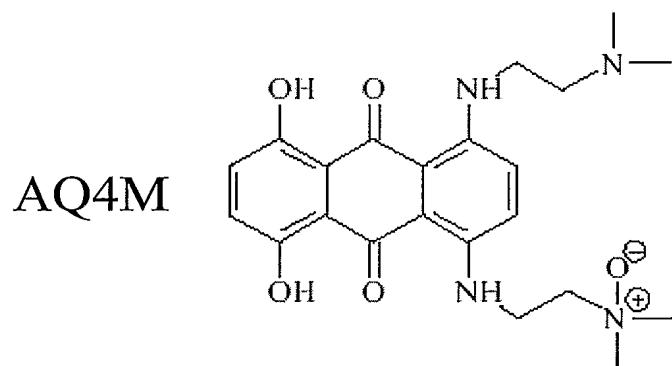


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This impurity can be formed by degradation of AQ4N, and more significantly shows an undesirable level of cytotoxicity, generally being higher than that of AQ4N itself. This level of cytotoxicity is to be avoided in a compound which is intended to be administered in the form of a relatively non-toxic prodrug.

15

Although the AQMN degradation is the predominant pathway, a further degradation product of AQ4N under acidic and neutral aqueous solution conditions is the mono-*N*-oxide, AQ4M:

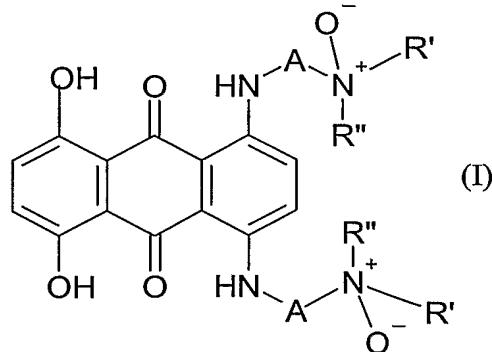


20

We have determined that the dihydrochloride salt AQ4N in aqueous solution typically gives a pH of around 2 to 3. For example, a 1.4 millimolar solution has a pH

of 2.4. We have now found that the above problem with impurity formation may be reduced or avoided by preparing, formulating and administering the compound so that upon dissolution in solution, the pH of the solution is in the range of 5 to 9.

Thus according to the present invention there is provided a compound of  
5 formula (I):



in which A is a C alkylene group with a chain length between NH and N(O)R'R'' of at least 2 carbon atoms and R' and R'' are each separately selected from C<sub>1-4</sub> alkyl groups and C<sub>2-4</sub> hydroxyalkyl and C<sub>2-4</sub> dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R'' together are a C<sub>2-6</sub> alkylene group which with the nitrogen atom to which R' and R'' are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

characterised in that the compound is formulated so that upon dissolution in  
15 aqueous solution the pH of the solution is in the range of 5 to 9.

Preferably the compound is formulated so that upon dissolution in aqueous solution the pH of the solution is in the range of 6 to 8.

Preferably the compound is formulated so that upon dissolution in aqueous solution at a concentration of between 0.1 and 100 mg/ml the pH of the solution is in  
20 the specified range.

The compounds (I) may be used in the form of a physiologically acceptable salt which will be an acid addition salt with an organic or inorganic acid. Preferably the physiologically acceptable acid has a pK<sub>a</sub> in the range of -3.0 (minus 3.0) to 9.0, and more preferably in the range of 2.0 to 9.0. More preferably the physiologically acceptable acid has a pK<sub>a</sub> in the range of 2.0 to 6.0.

Preferably the physiologically acceptable acid is selected from the group consisting of tartaric acid, malonic acid, dichloroacetic acid, citric acid, maleic acid, benzenesulfonic acid, pimelic acid and acetic acid.

More preferably the physiologically acceptable acid has a  $pK_a$  in the range of 5 3.0 to 6.0. The physiologically acceptable acid may especially be an organic acid, particularly an organic mono-, di- or tri-acid, and especially one selected from the group consisting of tartaric acid, citric acid, pimelic acid and acetic acid.

A in formula (I) may be branched but is conveniently a straight chain alkylene group, i.e. tetramethylene, especially trimethylene, or particularly ethylene.

10 R' and R" may also have a branched carbon chain but are conveniently straight chain whether they are alkyl groups or hydroxy-substituted alkyl groups. When R' or R" is a monohydroxyalkyl group this is conveniently substituted terminally and when R' or R" is a dihydroxyalkyl group this is conveniently substituted terminally by one of the hydroxy groups. When R' and R" are alkyl the preference is for a group of three 15 or especially two or one carbon atoms and when R' and R" are hydroxy-substituted alkyl the preference is for the alkyl group to be of three carbon atoms or, in the case of a monohydroxyalkyl group, alternatively of two carbon atoms. Examples of preferred individual groups R' and R" are  $\text{CH}_3$ ,  $\text{CH}_2\text{CH}_3$ ,  $\text{CH}_2\text{CH}_2\text{CH}_3$ ,  $\text{CH}_2\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ ,  $\text{CH}(\text{CH}_3)\text{CH}_2\text{OH}$  and  $\text{CH}_2\text{CHOHCH}_2\text{OH}$ .

20 R' and R" will more usually be identical.

Alternatively, as indicated, R' and R" together with the nitrogen atom to which they are attached may represent a heterocyclic group —N( $\text{CH}_2$ )<sub>n</sub> where n is 2 to 6, i.e. 25 aziridin-1-yl, azetidin-1-yl, pyrrolidin-1-yl, piperidin-1-yl and perhydroazepin-1-yl, the smaller groups such as azetidin-1-yl and especially aziridin-1-yl being of most interest.

Specific groups  $\text{NH}-\text{A}-\text{N}(\text{O})\text{R}'\text{R}''$  of particular interest are 30  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}_3)\text{C}_2\text{H}_5$ ,  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{C}_2\text{H}_5)_2$ ,  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}_2\text{CH}_2\text{OH})_2$ ,  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH})_2$ ,  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}(\text{CH}_3)\text{CH}_2\text{OH})_2$ ,  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}_2\text{CHOHCH}_2\text{OH})_2$  and especially  $\text{NH}-(\text{CH}_2)_2-\text{N}(\text{O})(\text{CH}_3)_2$ .

The physiologically acceptable salt when simply dissolved in aqueous solution will normally give a solution having a pH lower than the desired range. For example, the acetate salt of AQ4N in a 1.4 millimolar solution aqueous solution has a pH of 3.8. Thus preferably the compound is formulated in a mixture containing

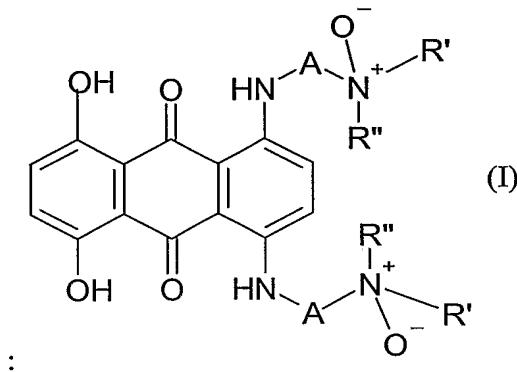
additional components so that upon dissolution in aqueous solution the pH of the solution is buffered to be in the range of 5 to 9.

5 A buffer is a solvated mixture of salt and acid, which oppose changes in pH when small amounts of acid and bases are added to the solution. Suitable buffers include sodium acetate buffer and sodium orthophosphate buffer.

According to a further aspect of the present invention there is provided an aqueous solution of a compound of formula (I), characterised in that the pH of the solution is in the range of 5 to 9.

10 Certain new salt forms may be used in the present invention. If they are salts of an acid having a  $pK_a$  higher than that of hydrochloric acid (approximately  $-6$ , i.e. minus 6), then they will be easier to formulate as a solid mixture containing additional components which buffer the pH when in aqueous solution, as compared to the dihydrochloride salt, since they are less acidic.

15 Thus according to a further aspect of the present invention there is provided a compound of formula (I):



in which A is a C alkylene group with a chain length between NH and N(O)R'R'' of at least 2 carbon atoms and R' and R'' are each separately selected from  $C_{1-4}$  alkyl groups and  $C_{2-4}$  hydroxyalkyl and  $C_{2-4}$  dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R'' together are a  $C_{2-6}$  alkylene group which with the nitrogen atom to which R' and R'' are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

20 characterised in that the compound is in the form of a salt with a physiologically acceptable acid having a  $pK_a$  in the range of  $-3.0$  (minus 3.0) to 9.0.

Preferably the physiologically acceptable acid has a  $pK_a$  in the range of 2.0 to 9.0. More preferably the physiologically acceptable acid has a  $pK_a$  in the range of 2.0 to 6.0.

5 The salts would generally be capable of being formed as anhydrous salts, and may be more stable as crystalline solids than to the dihydrochloride salt which would have water of crystallisation present. The salts would be easy to obtain in pure form.

10 The compounds (I) will be used in the form of a physiologically acceptable salt which will be an acid addition salt with an organic or inorganic acid. Physiologically acceptable acids having a  $pK_a$  in the range of -3.0 to 9.0 may be drawn from the following Table 1:

**Table 1:  $pK_a$  values of some common acids**

Free Acid or Base	$pK_a$ at 25 °C
Benzenesulfonic	-2.50
<i>p</i> -Toluenesulfonic	-1.34
Methanesulfonic	-1.2
Dichloroacetic	1.25
Maleic	2.00 ( $pK_{a1}$ )
Benzenehexacarboxylic (mellitic)	2.08 ( $pK_{a1}$ )
Phosphoric	2.12 ( $pK_{a1}$ )
Brucine tetrahydrate	2.30 ( $pK_{a1}$ )
Benzenepentacarboxylic	2.34 ( $pK_{a1}$ )
Glycine	2.34 ( $pK_{a1}$ )
Benzene-1,2,4,5-tetracarboxylic (pyromellitic)	2.43 ( $pK_{a1}$ )
Malonic	2.85 ( $pK_{a1}$ )
Phthalic	2.90
Salicylic	2.98
Benzene-1,2,3-tricarboxylic (hemimellitic)	2.98 ( $pK_{a1}$ )
Tartaric	3.02 ( $pK_{a1}$ )
Fumaric	3.03 ( $pK_{a1}$ )
Glycylglycine	3.06
Cyclopentanetetra-1,2,3,4-carboxylic	3.07 ( $pK_{a1}$ )
<i>o</i> -Phthalic	3.10 ( $pK_{a1}$ )
Citric	3.13 ( $pK_{a1}$ )
Benzene-1,2,4,5-tetracarboxylic (pyromellitic)	3.13 ( $pK_{a1}$ )
Benzene-1,3,5-tricarboxylic (trimesic)	3.16 ( $pK_{a1}$ )
Glucuronic	3.18
Dimethylmalonic	3.29 ( $pK_{a1}$ )
Mandelic	3.36
Butane-1,2,3,4-tetracarboxylic	3.36 ( $pK_{a1}$ )
Malic	3.40 ( $pK_{a1}$ )
1,1-Cyclohexanediacetic	3.52 ( $pK_{a1}$ )
2-Methylpropane-1,2,3-tricarboxylic	3.53 ( $pK_{a1}$ )

Free Acid or Base	pK <sub>a</sub> at 25 °C
Hippuric	3.64
Propane-1,2,3-tricarboxylic (tricarballylic)	3.67 (pK <sub>a1</sub> )
Formic	3.75
Gluconic	3.76
3,3-Dimethylglutaric	3.79 (pK <sub>a1</sub> )
1,1-Cyclopentanedicetic	3.82 (pK <sub>a1</sub> )
Itaconic	3.84 (pK <sub>a1</sub> )
Lactic	3.86
Barbituric	3.98
Ascorbic	4.10 (pK <sub>a1</sub> )
2,2-Dimethylsuccinic	4.11 (pK <sub>a1</sub> )
Succinic	4.19 (pK <sub>a1</sub> )
Benzoic	4.20
3,6-Endomethylene-1,2,3,6-tetrahydronphthalic "EMTA"	4.30 (pK <sub>a1</sub> )
2,2-Dimethylglutaric	4.31 (pK <sub>a1</sub> )
Pimelic	4.48
Acetic	4.75
Sorbic	4.76
<i>n</i> -Butyric	4.82
Propionic	4.87
Malic	5.05 (pK <sub>a2</sub> )
Pyridine	5.23
Hydroxylamine	6.03
Edetic	6.16 (pK <sub>a3</sub> )
Carbonic	6.35 (pK <sub>a1</sub> )
Citric	6.40 (pK <sub>a3</sub> )
Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane "BIS-TRIS"	6.46
Imidazole	7.00
2-(Aminoethyl)trimethylammonium chloride "CHOLAMINE"	7.10
Phosphoric	7.21 (pK <sub>a2</sub> )
2-Hydroxyethyliminotris(hydroxymethyl)methane "MONO-TRIS"	7.83
4-(2-Hydroxyethyl)-1-piperazinepropane sulfonic "EPPS"	8.00

Preferably the physiologically acceptable acid is selected from the group consisting of tartaric acid, malonic acid, dichloroacetic acid, citric acid, maleic acid, benzenesulfonic acid, pimelic acid and acetic acid.

5 More preferably the physiologically acceptable acid has a pK<sub>a</sub> in the range of 3.0 to 6.0. The physiologically acceptable acid may especially be an organic acid, particularly an organic mono-, di- or tri-acid, and especially one selected from the group consisting of tartaric acid, citric acid, pimelic acid and acetic acid.

10 A in formula (I) may be branched but is conveniently a straight chain alkylene group, i.e. tetramethylene, especially trimethylene, or particularly ethylene.

R' and R" may also have a branched carbon chain but are conveniently straight chain whether they are alkyl groups or hydroxy-substituted alkyl groups. When R' or R" is a monohydroxyalkyl group this is conveniently substituted terminally and when R' or R" is a dihydroxyalkyl group this is conveniently substituted terminally by one of the hydroxy groups. When R' and R" are alkyl the preference is for a group of three or especially two or one carbon atoms and when R' and R" are hydroxy-substituted alkyl the preference is for the alkyl group to be of three carbon atoms or, in the case of a monohydroxyalkyl group, alternatively of two carbon atoms. Examples of preferred individual groups R' and R" are CH<sub>3</sub>, CH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>OH, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH, CH(CH<sub>3</sub>)CH<sub>2</sub>OH and CH<sub>2</sub>CHOHCH<sub>2</sub>OH.

10 R' and R" will more usually be identical.

Alternatively, as indicated, R' and R" together with the nitrogen atom to which they are attached may represent a heterocyclic group —N(CH<sub>2</sub>)<sub>n</sub> where n is 2 to 6, i.e. aziridin-1-yl, azetidin-1-yl, pyrrolidin-1-yl, piperidin-1-yl and perhydroazepin-1-yl, 15 the smaller groups such as azetidin-1-yl and especially aziridin-1-yl being of most interest.

Specific groups NH—A—N(O)R'R" of particular interest are  
NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>3</sub>)C<sub>2</sub>H<sub>5</sub>, NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>,  
NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>, NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>,  
20 NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH(CH<sub>3</sub>)CH<sub>2</sub>OH)<sub>2</sub>, NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>2</sub>CHOHCH<sub>2</sub>OH)<sub>2</sub> and especially NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>3</sub>)<sub>2</sub>.

The salt with a physiologically acceptable acid may be prepared by any conventional means, for example by reaction of the organic base (I) with the appropriate inorganic or organic acid, usually by simple admixture in solution. The 25 acid addition salts are generally crystalline solids which are relatively soluble in water, methanol, ethanol and similar solvents. One salt form may also be converted into another by chromatography using a column which has been pre-treated with the desired physiologically acceptable acid.

The compounds (I) may be formulated with a physiologically acceptable 30 diluent or carrier for use as pharmaceuticals for both veterinary and particularly human use by a variety of methods. For instance, they may be applied as a composition incorporating a liquid diluent or carrier, for example an aqueous solution, suspension or emulsion, which may often be employed in injectable form for parenteral administration and therefore may conveniently be sterile and pyrogen free.

Oral administration may also be used and although compositions for this purpose may incorporate a liquid diluent or carrier, it is more usual to use a solid, for example a conventional solid carrier material such as starch, lactose, dextrin or magnesium stearate. Such solid compositions may take the form of powders but are more 5 conveniently of a formed type, for example as tablets, cachets, or capsules. Alternative, more specialized types of formulation include liposomes and nanoparticles.

Other types of administration than by injection or through the oral route which are of use in both human and veterinary contexts include the use of suppositories or 10 pessaries. Another form of pharmaceutical composition is one for buccal or nasal administration or alternatively drops for administration into the eye which may conveniently contain a sterile liquid diluent or carrier. Other formulations for topical administration include lotions, ointments, creams, gels and sprays.

Compositions may be formulated in unit dosage form, i.e. in the form of 15 discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

Whilst the dosage of the compound used will vary according to the activity of the particular compound and the condition being treated, it may be stated by way of guidance that a dosage selected in the range from 25–500 mg/m<sup>2</sup> per day, particularly in the range from 50–300 mg/m<sup>2</sup> per day, will often be suitable although higher doses 20 than this, for example in the range from 25–750 mg/m<sup>2</sup> per day, or even doses up to 1200 mg/m<sup>2</sup>, may be considered in view of the lower level of toxic side effects obtained with the compounds (I). This dosage regime may be continued for however many days is appropriate to the patient in question, the daily dosages being divided into several separate administrations if desired. Thus, for example, in the case of 25 conditions such as advanced breast cancer, non-Hodgkin's lymphoma and hepatoma, treatment for one day followed by a repeated dose after an interval, such as 21 days, may be appropriate whilst for the treatment of acute non-lymphocytic leukaemia, treatment over 5 consecutive days may be more suitable. Alternatively, single administrations spaced by several days, for example one dose every two or three 30 weeks, may be used.

The compounds (I) are of particular value for the treatment of cancer in warm blooded animals including humans. The compounds are of interest in relation to the treatment of solid tumours, such as various forms of sarcoma and carcinoma, and also for disseminated tumours such as leukaemias. Areas of particular interest are the

treatment of breast cancer, lung cancer, prostate cancer, pancreatic cancer, and oesophageal cancer, and the treatment of non-Hodgkin's lymphoma and acute non-lymphocytic leukaemia. In the treatment of cancer, parenteral and sometimes topical administration is often of particular interest. Moreover, it may be advantageous to use  
5 the compounds (I) in a combined treatment, given separately or together in the same composition, with other anti-cancer agents, such as mitotic inhibitors, for example vinblastine; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; other antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating antibiotics, for example adriamycin and bleomycin;  
10 enzymes, for example asparaginase; topoisomerase inhibitors, for example etoposide and biological response modifiers, for example interferon. The compounds (I) may also be used in combined treatment with radiation therapy of the tumour.

The present invention thus includes a method for aiding regression and palliation of cancer which comprises administering to a patient a therapeutically effective amount of a compound (I) as defined hereinbefore.  
15

In addition to their anti-cancer use the compounds (I) are of interest for various other pharmaceutical applications in view of their activity as chelating agents.

The invention is illustrated by the following Examples in which—

Figure 1 shows the first derivative of pH *versus* pH in a solution of AQ4N  
20 dihydrochloride;

Figure 2 shows the first derivative of pH *versus* NaOH molar equivalence under the same conditions;

Figure 3 shows the increase in AQMN over incubation time in 5 mg/ml solutions incubated at 40 °C for 14 days.

Figure 4 shows the increase in AQMN over incubation time in 5 mg/ml solutions incubated at 40 °C for 63 days;  
25

Figure 5 shows the decrease in AQ4N over incubation time in 5 mg/ml solutions incubated at 40 °C for 14 days;

Figure 6. shows the decrease in AQ4N over incubation time in 5 mg/ml  
30 solutions incubated at 40 °C for 63 days;

Figure 7 shows the formal method used to prepare salts of AQ4N; and

Figure 8 shows the organic acids used to prepare salts of AQ4N.

## EXAMPLES

### Example 1: Demonstration of the instability of AQ4N dihydrochloride— physico-chemical properties of AQ4N

Changes in the pH of a solution of AQ4N dihydrochloride were monitored to 5 demonstrate the degradation of AQ4N into AQMN. The pH curves are shown in Figures 1 and 2. Figure 1 shows a clear dissociation at between pH 7.7 and pH 9.4, and this equates to the dissociation events shown in Figure 2 at approximately 2 molar equivalence. A low pH dissociation event can be observed, speculatively assigned to a pH between 4.1 and 4.6 where the molar equivalence is between 0.95 10 and 1.15.

### Example 2: Demonstration of the cytotoxicity of AQMN

The toxicity of a pure sample (99.3%) in the P388 system of AQ4N and AQMN were determined and the results obtained are presented in Table 2.

**Table 2: AQ4N and AQMN cytotoxicity values**

Compound	IC <sub>50</sub> P388 (nM)	Relative toxicity (normalised to AQ4N)
AQ4N	410	1.0
AQMN	77	5.2

15

Based on these data, AQMN has a cytotoxicity which is at least 5 times greater than that of AQ4N in the same system. The “greater than” modifier is required since all samples of AQ4N contain substantial percentages of AQMN, which will affect the toxicity result.

20

### Example 3: Demonstration of the instability of AQ4N dihydrochloride in solution—accumulation of AQMN

The degradation of AQ4N was investigated using 5 mg/ml solutions of AQ4N at a pH of 2.4, 4.5 and 6.8, which equated to water, 20 mM sodium acetate buffer and 20 mM sodium orthophosphate buffer, respectively. The primary degradation 25 pathway of AQ4N is its conversion to AQMN. The increase in AQMN concentration

in 5 mg/ml solutions incubated at 40 °C over an intermediate time period (14 days) is shown in Figure 3.

The degradation rates equate to a 0.84% (w.r.t. AQ4N), 0.19% (w.r.t. AQ4N) and 0.02% (w.r.t. AQ4N) increase in AQMN content per day under these conditions.

Using linear regression and cross correlating with the known quantity of AQMN in the material used as a standard these data indicate the accumulation rates of AQMN presented in Table 3.

**Table 3: Accumulation of AQMN**

	H <sub>2</sub> O	Acetate	PO <sub>4</sub>
AQMN increase per day	0.84%	0.19%	0.02%

A similar trend was observed in the data after 63 days, as shown in Figure 4. The rate of accumulation (calculated in the same way as above) shows that in the phosphate buffer AQMN increases by 0.6% per month.

**Example 4: Demonstration of the instability of AQ4N dihydrochloride in solution—degradation of AQ4N**

The effect of pH on the stability of AQ4N was determined by investigating AQ4N degradation in different solutions. The solutions chosen were again distilled water, 20 mM sodium acetate buffer (pH = 4) and 20 mM sodium phosphate buffer (pH = 7). After preparation of the 5 mg/ml AQ4N buffered solutions the pH was corrected to the required pH of the buffer. The final pHs were 2.4, 4.5 and 6.8 for the distilled water, 20 mM sodium acetate buffer and 20 mM sodium phosphate buffer, respectively. The samples were incubated at 40 °C and sampled at regular intervals. Assay was carried out by sample dilution followed by HPLC analysis.

After 14 days an interim analysis was carried out with the data being shown in Figure 5.

The solutions were further incubated at 40 °C for a total of 63 days. The final graph is shown in Figure 6. The values obtained for the AQ4N contents are consistent with the quantities of AQ4N weighed into the original samples (within experimental error).

**Example 5: Synthesis of some organic acid salts of AQ4N**

A series of organic acid addition salts of AQ4N were prepared as shown formally in Figure 7 and their stability with respect to AQMN formation was determined.

5 The choice of method for the synthesis of AQ4N salts was limited by the high solubility of AQ4N free base in polar solvents. Synthesis of malonic, citric, tartaric acid addition salts of AQ4N was initially attempted by adding an aqueous solution (1M) of the selected organic acid to a solution of AQ4N free base in a polar solvent/aqueous mixture such as H<sub>2</sub>O/CH<sub>3</sub>CN (1:1) or H<sub>2</sub>O with a minimal amount of  
10 DMSO. After stirring for 2 hours, the reaction mixtures were cooled to 4 °C to facilitate precipitation. However, no solid was observed after 7 days. Subsequent work used MeOH as a solvent that readily to dissolve both AQ4N and all organic acids. In all cases except in the preparation of benzenesulfonic acid, the AQ4N was added to 20-fold excess of organic acid and these mixtures were left for 14–24 hours  
15 at room temperature and then at –10 °C to aid precipitation. If very little or no solid material was observed, diethyl ether was added to the mixture to facilitate precipitation.

**Example 6: AQ4N di-benzenesulfonate**

AQ4N (36 mg, 0.081 mmol) was dissolved in MeOH (5 mL) and stirred for 5  
20 minutes. Benzenesulfonic acid (25.66 mg, 0.162 mmol) dissolved in 1 mL MeOH was added to the stirred solution of AQ4N. The reaction mixture was stirred for half an hour, and the precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 16 hours. Yield: 45 mg, 76 %. Anal. calcd. for C<sub>34</sub>H<sub>40</sub>N<sub>4</sub>O<sub>12</sub>S: C, 56.04; H, 5.53; N, 7.69. Found: C, 25 55.80; H, 5.35; N, 9.30.

**Example 7: AQ4N di-dichloroacetate**

AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Dichloroacetic acid (38 µl, 0.46 mmol) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by  
30 16 hours at –10 °C. The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 16

hours. Yield: 7.4 mg, 46 %. Anal. calcd. for  $C_{26}H_{32}N_4O_{10}Cl_4$ : C, 44.46; H, 4.59; N, 7.98. Found: C, 44.58; H, 4.61; N, 7.91.

#### Example 8: AQ4N di-maleate

5 AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Maleic acid (53.4 mg, 0.46 mmol) in MeOH (1 mL) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 16 hours at  $-10^{\circ}\text{C}$ . The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 16 hours. Yield: 7 mg, 47 %. Anal. calcd. for  $C_{30}H_{36}N_4O_{14}$ : C, 53.24; H, 5.32; N, 8.28. Found: C, 53.11; H, 5.46; N, 8.20.

10

#### Example 9: AQ4N di-malonate

15 AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Malonic acid (47.9 mg, 0.46 mmol) in MeOH (1 mL) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 24 hours at  $-10^{\circ}\text{C}$ . The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 16 hours. Yield: 8.2 mg, 55 %. Anal. calcd. for  $C_{28}H_{36}N_4O_{14}$ : C, 51.53; H, 5.56; N, 8.59. Found: C, 51.67; H, 5.56; N, 8.74.

#### Example 10: AQ4N di-tartrate

20 AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Tartaric acid (69.1 mg, 0.46 mmol) in MeOH (1 mL) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 16 hours at  $-10^{\circ}\text{C}$ . The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 24 hours. Yield: 8.5 mg, 50 %. Anal. calcd. for  $C_{30}H_{40}N_4O_{18}$ : C, 48.39; H, 5.41; N, 7.52. Found: C, 48.34; H, 5.41; N, 7.67.

25

#### Example 11: AQ4N di-citrate

30 AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Citric acid (88.4 mg, 0.46 mmol) in MeOH (1 mL) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 16 hours at  $-10^{\circ}\text{C}$ . Ether (2 mL) was added to the reaction mixture and

set aside at  $-10^{\circ}\text{C}$  for another 3 hours. The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 24 hours. Yield: 7.1 mg, 37 %. Anal. calcd. for  $\text{C}_{34}\text{H}_{44}\text{N}_4\text{O}_{20}$ : C, 49.28; H, 5.10; N, 6.65. Found: C, 49.59; H, 5.09; N, 6.64.

5

**Example 12: AQ4N di-lactate**

AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Lactic acid (35  $\mu\text{l}$ , 0.46 mmol) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 16 hours at  $-10^{\circ}\text{C}$ . Ether (2 mL) was added to the reaction mixture and set aside at  $-10^{\circ}\text{C}$  for 10 another 3 hours. The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 24 hours. Yield: 4.5 mg, 31 %. Anal. calcd. for  $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_{12}$ : C, 53.84; H, 6.45; N, 8.97. Found: C, 51.54; H, 6.24; N, 8.89.

**Example 13: AQ4N di-pimelate**

15 AQ4N (10 mg, 0.023 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Pimelic acid (73.7 mg, 0.46 mmol) in MeOH (1 mL) was added to the stirred solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 14 hours at  $-10^{\circ}\text{C}$ . The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in 20 a desiccator for 24 hours. Yield: 8.2 mg, 47 %. Anal. calcd. for  $\text{C}_{36}\text{H}_{52}\text{N}_4\text{O}_{14}$ : C, 56.80; H, 6.80; N, 7.34. Found: C, 56.49; H, 6.41; N, 8.46.

**Example 14: AQ4N di-acetate**

AQ4N (70 mg, 0.158 mmol) was dissolved in MeOH (1 mL) and stirred for 5 minutes. Acetic acid (183  $\mu\text{l}$ , 3.16 mmol) in MeOH (1 mL) was added to the stirred 25 solution of AQ4N. The reaction mixture was stirred for 1 hour at room temperature followed by 18 hours at  $-10^{\circ}\text{C}$ . Ether (3 mL) was added to the reaction mixture and set aside at  $-10^{\circ}\text{C}$  for another 3 hours. The precipitated solid was isolated by filtration. The dark blue solid was dried over phosphorous pentoxide under vacuum in a desiccator for 24 hours. Yield: 68 mg, 76 %. Anal. calcd. for  $\text{C}_{26}\text{H}_{36}\text{N}_4\text{O}_{10}$ : C, 30 55.31; H, 6.43; N, 9.92. Found: C, 55.28; H, 6.57; N, 9.91.

**Example 15: Determination of the pH of organic acid salts of AQ4N in aqueous solution at 37 °C**

Stock solutions of individual AQ4N salts were prepared by transferring about 1 mg, accurately weighed, and diluting with water to obtain solutions of 1.4 mM. The 5 solution was vortexed for 3 min using a Fisons WhirlMixer™ (Loughborough, Leicestershire, UK). All AQ4N salt solutions were prepared on the same day as determination of the pH value of the solutions, which was done using a digital pH meter (calibrated at 21±1 °C with standard buffers of pH 7.00 and 4.00 from BDH (UK)).

10 Table 4 and Figure 7 shows the nine organic acids used to prepare salts of AQ4N in order of increasing  $pK_{a1}$  values.

**Table 4:  $pK_{a1}$  of acids used to prepare salts of AQ4N and resulting pH of aqueous solutions**

Acid used to prepare AQ4N disalt	$pK_{a1}$	pH <sup>a</sup>	Acid used to prepare AQ4N disalt	$pK_{a1}$	pH <sup>a</sup>
Hydrochloric	~ -6	2.4	Tartaric	3.02	2.8
Benzenesulfonic	-2.5	2.5	Citric	3.13	3.3
Dichloroacetic	1.25	2.4	DL-Lactic	3.86	3.5
Maleic	2	2.6	Pimelic	4.48	3.8
Malonic	2.85	2.8	Acetic	4.75	3.8

15

15

<sup>a</sup> measured pH of AQ4N di-salt solutions (1.4 mM).

20  $pK_a$  data are from "IUPAC Handbook of Pharmaceutical Salts, Properties, selection and Use", P. Heinrich Stahl, Camille G. Wermuth (Eds) VHCA Verlag Helvetica Chimica Acta, Zurich & Wiley-VCH, Weinheim (joint publ.) 2002.

For comparison the  $pK_a$  for hydrochloric acid is also included. The organic acids used to prepare AQ4N salts in this study were:

- Mono-acid: benzenesulfonic, dichloroacetic, lactic, acetic
- Di-acid: maleic, malonic, tartaric, pimelic
- 25 • Tri-acid: citric

The pK<sub>a</sub>s of these acids are a measure of the degree of ionisation of each acid moiety and hence will reflect their acidity as measured by pH. Table 4 shows that the measured pH of the AQ4N salt (1.4 mM) solutions was as low as pH 2.4 & 2.5 for benzenesulfonate and dichloroacetate salts respectively and pH 3.8 for acetate and 5 pimelate salts.

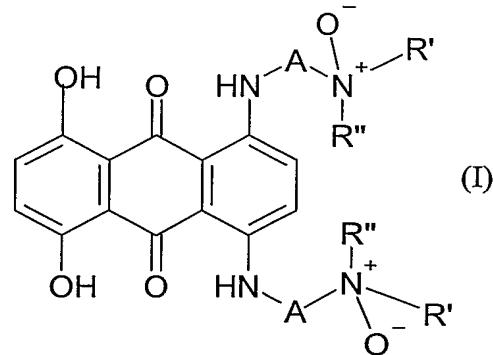
**Example 16: Determination of the purity of organic acid salts of AQ4N in aqueous solution at 37 °C**

The chromatographic separation of AQ4N salt solutions were performed using a gradient mobile phase and a HiChrom HIRPB (250 mm x 4.6 mm, 5 µm) column 10 (HiChrom, Agilent, Reading, UK, P/N HIRPB-6294) housed into a Flatron Column Heater system at 24 °C. The mobile phase used for the gradient separation of AQ4N salts consisted of solvent system A (5% acetonitrile 95% ammonium formate buffer (0.05M, pH 3.6) and solvent system B (50% acetonitrile 50% ammonium formate (0.05M, pH 3.6). The pH was adjusted to 3.6 with formic acid. The gradient was run 15 from 20% mobile phase A to 95% mobile phase B over 55 minutes. A flow rate of 1.3 ml min<sup>-1</sup> was used for all separations. Data were collected using  $\lambda = 612$  nm and the data were processed using Waters Millennium<sup>32TM</sup> software.

Freshly prepared solutions of the prepared AQ4N salts (1.4 mM) were chromatographed within 30 min of dissolution. All the AQ4N salts were shown to be 20 predominantly AQ4N (greater than 99%) with less than 1% the mono-N-oxide AQ4M or AQMN.

## CLAIMS

1. A compound of formula (I):



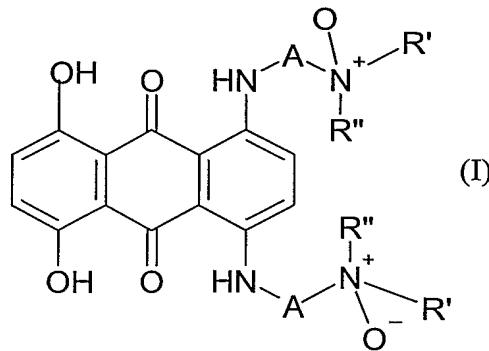
5 in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C<sub>1-4</sub> alkyl groups and C<sub>2-4</sub> hydroxyalkyl and C<sub>2-4</sub> dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C<sub>2-6</sub> alkylene group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

10 characterised in that the compound is formulated so that upon dissolution in aqueous solution the pH of the solution is in the range of 5 to 9.

15 2. A compound as claimed in claim 1 characterised in that the compound is formulated so that upon dissolution in aqueous solution the pH of the solution is in the range of 6 to 8.

20 3. A compound as claimed in claim 1 or claim 2 characterised in that the compound is used in the form of a salt with an physiologically acceptable acid having a pK<sub>a</sub> in the range of -3.0 (minus 3.0) to 9.0.

4. A compound of formula (I):



in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C<sub>1-4</sub> alkyl groups and C<sub>2-4</sub> hydroxyalkyl and C<sub>2-4</sub> dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C<sub>2-6</sub> alkylene group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

characterised in that the compound is in the form of a salt with a physiologically acceptable acid having a pK<sub>a</sub> in the range of -3.0 (minus 3.0) to 9.0.

5. A compound as claimed in claim 3 or 4 characterised in that the physiologically acceptable acid has a pK<sub>a</sub> in the range of 2.0 to 9.0.

15 6. A compound as claimed in claim 5 characterised in that the physiologically acceptable acid has a pK<sub>a</sub> in the range of 2.0 to 6.0.

7. A compound as claimed in claim 6 characterised in that the physiologically acceptable acid has a pK<sub>a</sub> in the range of 3.0 to 6.0.

20 8. A compound as claimed in claim 3 characterised in that the physiologically acceptable is an organic mono-, di- or tri-acid.

25 9. A compound as claimed in claim 3 or 4 characterised in that the physiologically acceptable selected from the group consisting of tartaric acid, malonic acid, dichloroacetate acid, citric acid, maleic acid, benzenesulfonic acid, pimelic acid and acetic acid.

10. A compound as claimed in any preceding claim characterised in that A is a straight chain alkylene group.

5 11. A compound as claimed in any preceding claim characterised in that A is ethylene.

12. A compound as claimed in any preceding claim characterised in that R' and R" are straight chain alkyl groups or hydroxy-substituted alkyl groups.

10

13. A compound as claimed in claim 12 characterised in that R' and R" are each CH<sub>3</sub> or CH<sub>2</sub>CH<sub>3</sub>.

14. A compound as claimed in claim 13 characterised in that each group of  
15 formula NH—A—N(O)R'R" is group of formula NH—(CH<sub>2</sub>)<sub>2</sub>—N(O)(CH<sub>3</sub>)<sub>2</sub>.

20

15. A compound as claimed in any preceding claim characterised in that the compound is formulated in a mixture containing additional components so that upon dissolution in aqueous solution the pH of the solution is buffered to be in the range of 5 to 9.

16. An aqueous solution of a compound as claimed in any preceding claim, characterised in that the pH of the solution is in the range of 5 to 9.

25

17. A pharmaceutical composition comprising a compound of formula (I) as defined in any of claims 1 to 14 together with a physiologically acceptable diluent or carrier.

30

18. A compound of formula (I) as defined in any of claims 1 to 15 for use in therapy.

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Fig.1.

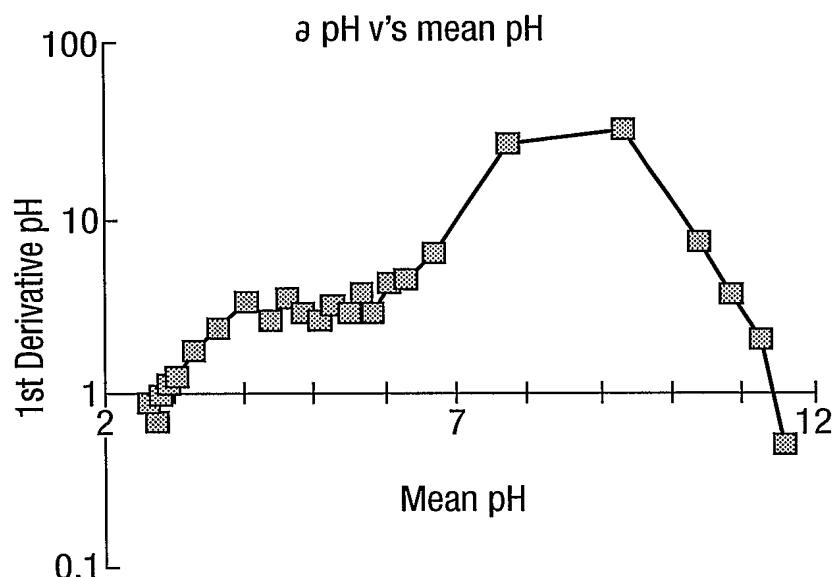
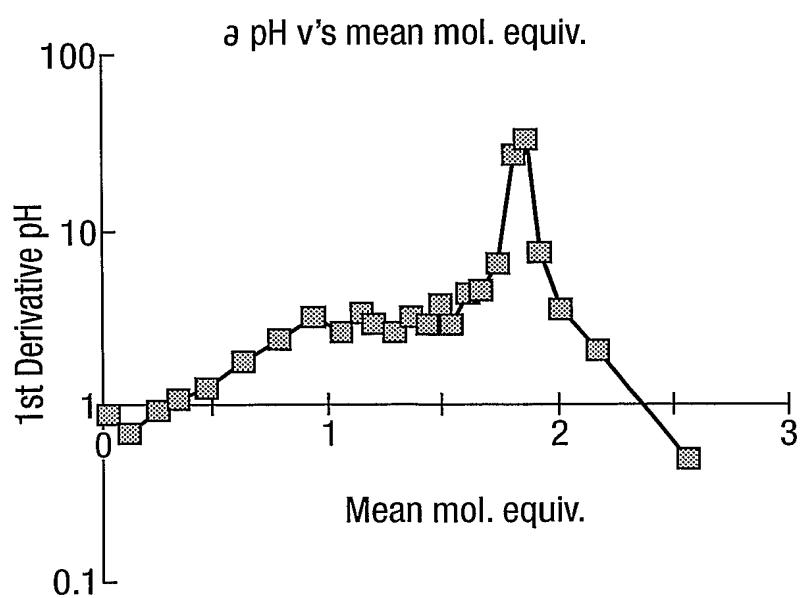


Fig.2.



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Fig.3.

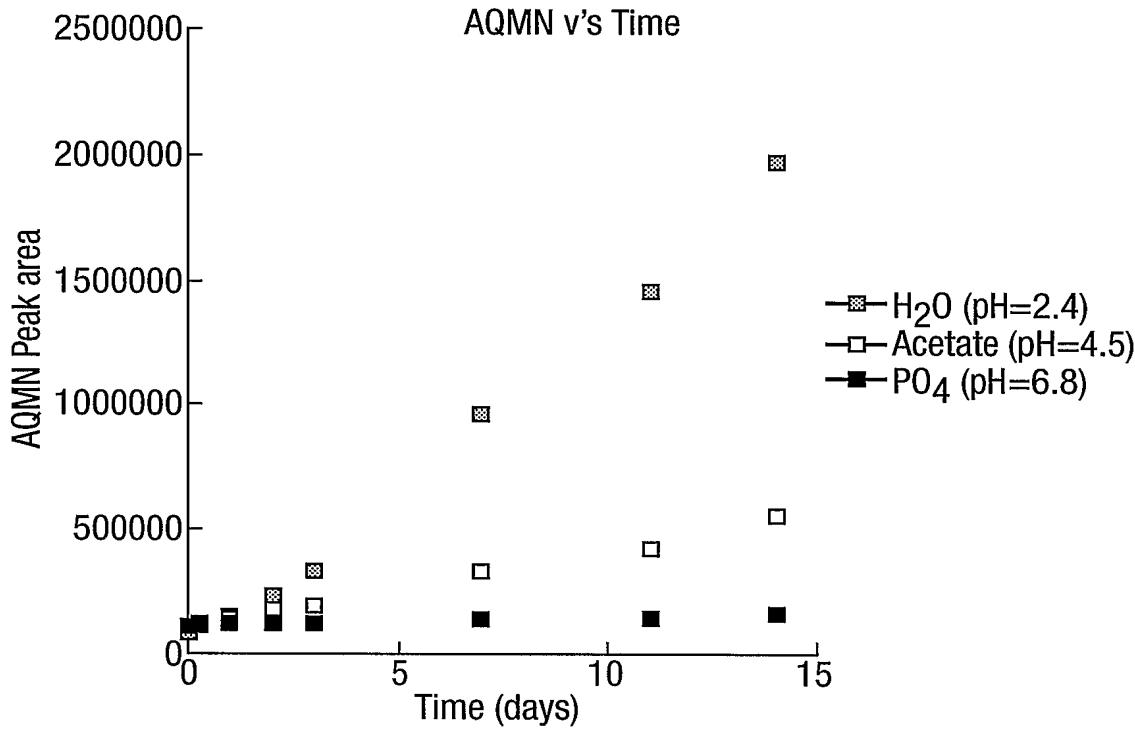
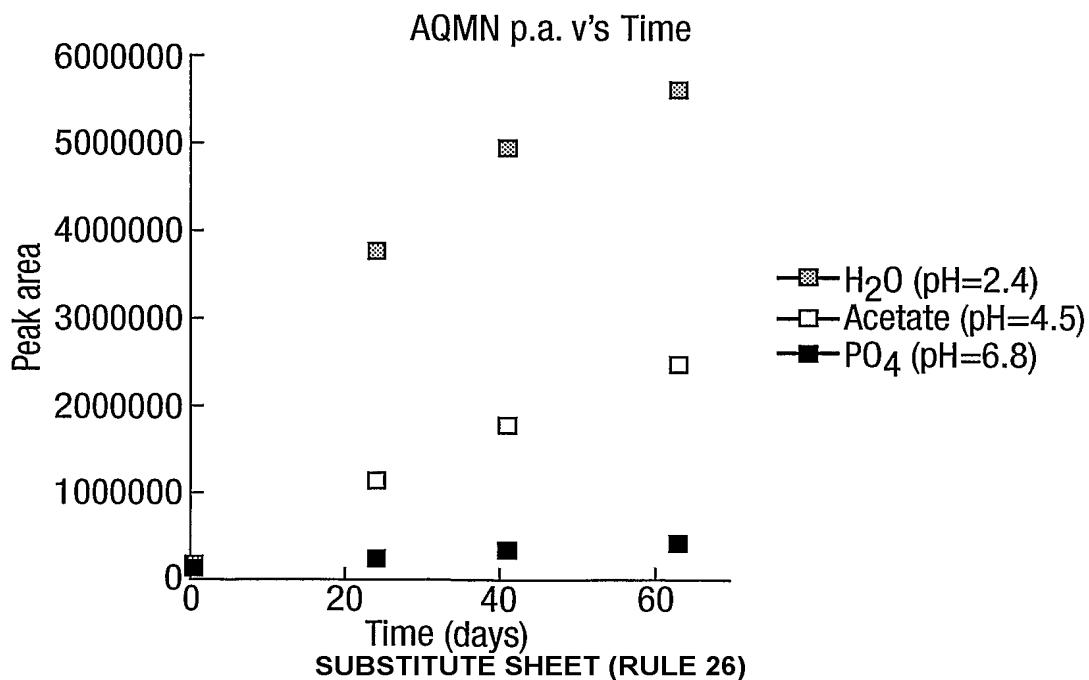


Fig.4.



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Fig.5.

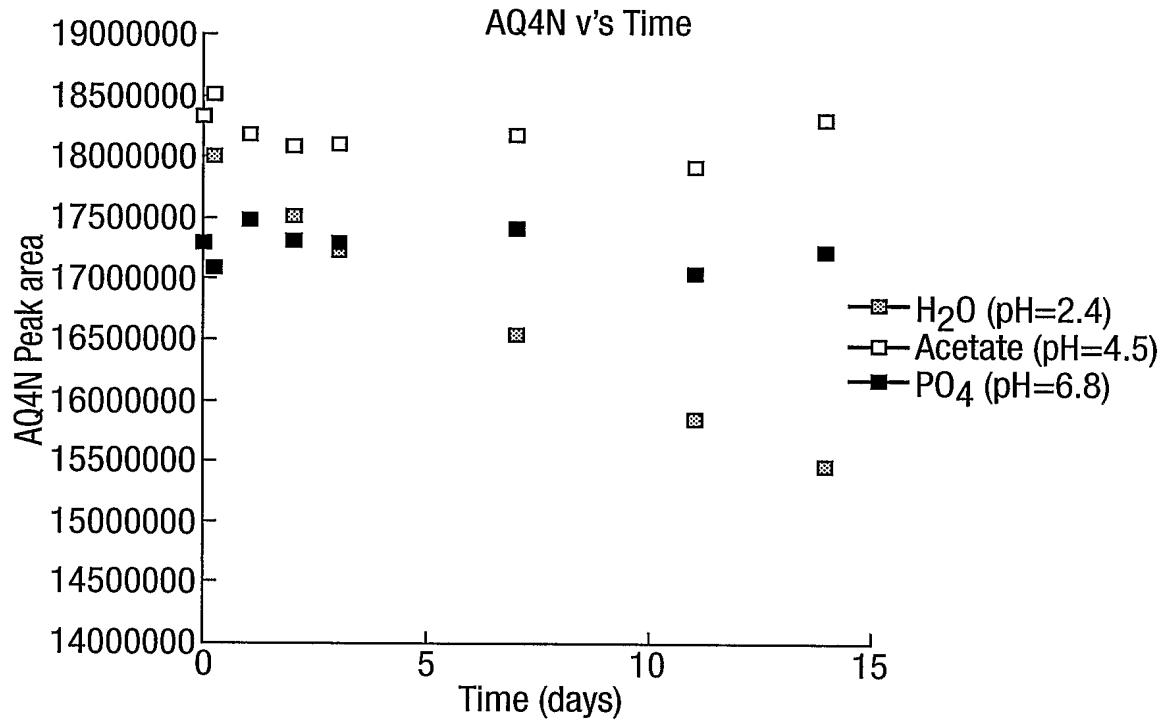
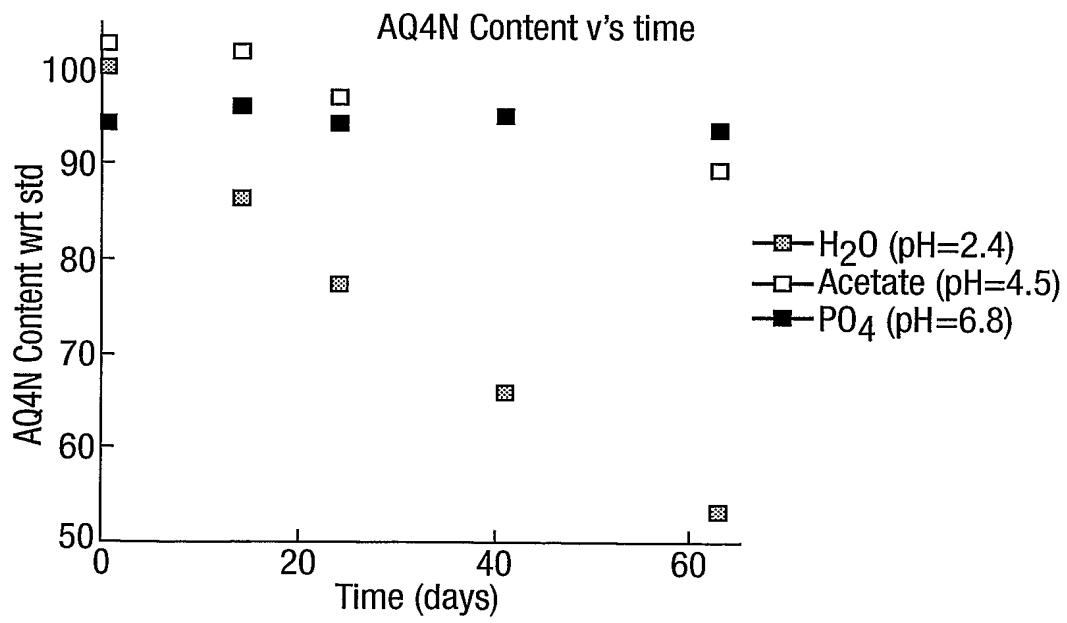


Fig.6.



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Fig.7.

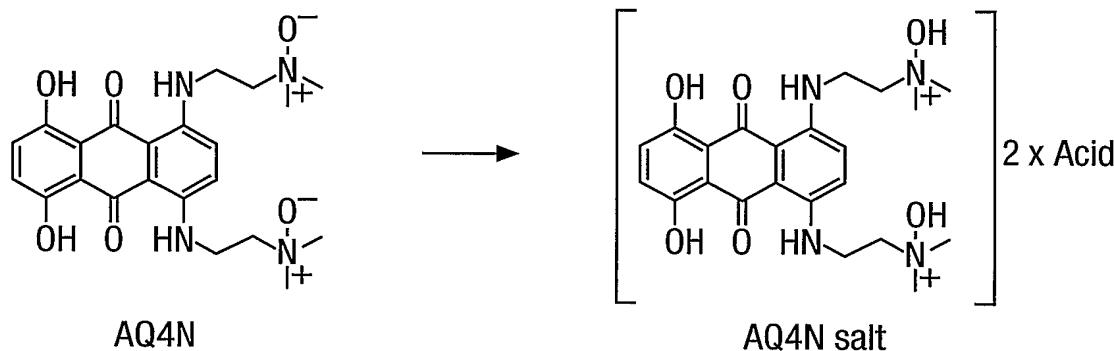
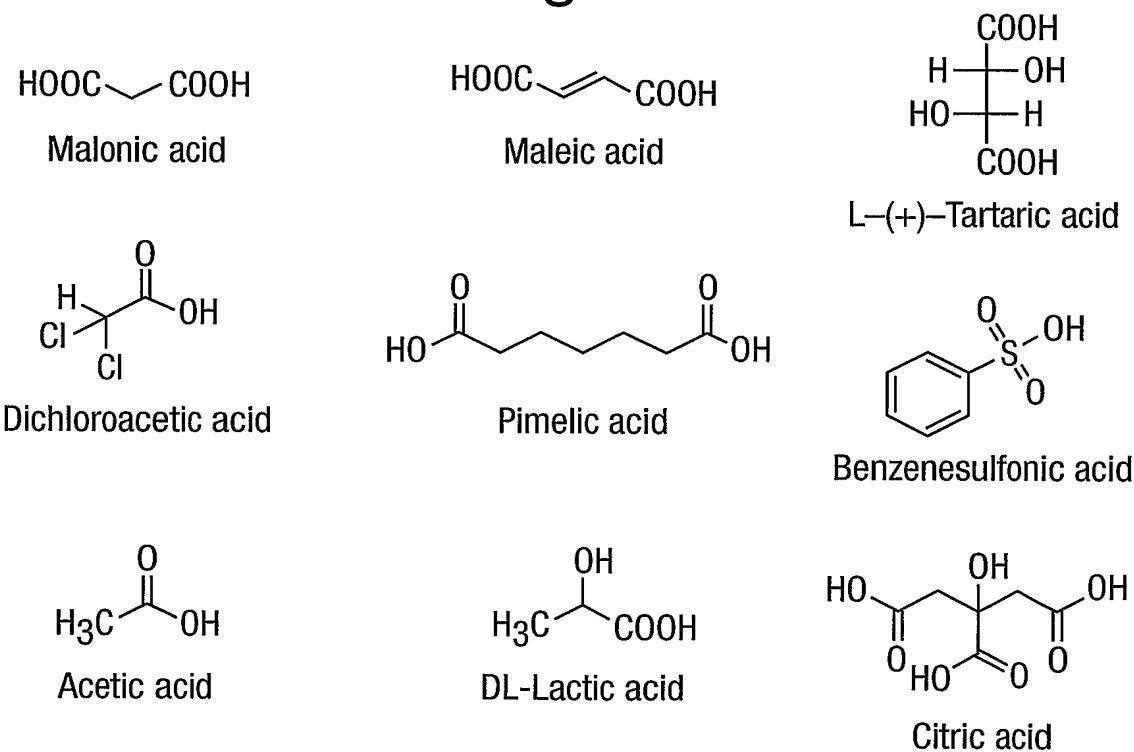


Fig.8.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/01110

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 C07C291/04 A61K47/02 A61K47/12

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 05194 A (BTG) 3 February 2000 (2000-02-03) cited in the application page 11, line 31 -page 12, line 24; claims ---	1,4
A	HO H. LEE ET AL.: "A Large-scale Synthesis of the Bioreductive Drug 1,4-Bis{[2-(dimethylamino)ethyl]amino}-5,8 -dihydroxyanthracene-9,10-dione bis-N-oxide" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1., 1999, pages 2755-2758, XP002244552 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0300-922X cited in the application page 2758, column 1, line 10 - line 28 ---	1,4 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2003	30/06/2003
Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Zervas, B

**INTERNATIONAL SEARCH REPORT**

International Application No  
PCT/GB 03/01110

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 05824 A (NATIONAL RESEARCH DEVELOPMENT) 2 May 1991 (1991-05-02) cited in the application claims; examples -----	1, 4

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/GB 03/01110

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0005194	A 03-02-2000	CA	2337070 A1	03-02-2000
		EP	1097125 A1	09-05-2001
		WO	0005194 A1	03-02-2000
		JP	2002521357 T	16-07-2002
		US	6320063 B1	20-11-2001
WO 9105824	A 02-05-1991	AT	101181 T	15-02-1994
		AU	634125 B2	11-02-1993
		AU	6539590 A	16-05-1991
		CA	2038934 A1	14-04-1991
		DE	69006482 D1	17-03-1994
		DE	69006482 T2	11-05-1994
		DK	450021 T3	07-03-1994
		EP	0450021 A1	09-10-1991
		ES	2062558 T3	16-12-1994
		WO	9105824 A1	02-05-1991
		GB	2237283 A , B	01-05-1991
		JP	2854971 B2	10-02-1999
		JP	4502166 T	16-04-1992
		NZ	235658 A	23-12-1992
		PT	95584 A , B	13-09-1991
		US	5132327 A	21-07-1992
		ZA	9008178 A	24-06-1992